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# Potential of Skin Melanocytes for Developing Acquired Proliferative Melanocytic Lesions: Cellular and Molecular Aspects

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Several epidemiological studies have shown [1-4] that the potential of human skin melanocytes for developing melanocytic tumors in response to mitogenic stimuli differs according to anatomical location. However, despite recent advances, the proliferative responses of different human skin melanocytes remain unknown. The complex, multifactorial, and heterogeneous process [5] of melanocyte differentiation occurs throughout life, from embryonic development to adulthood, and it appears to involve both genetic [5,6] and environmental [1-6] factors.

Furthermore, an improved understanding of the cellular and molecular mechanisms of melanocytes may provide important information on the development of acquired melanocytic tumors, although research in this field is still in its early stages.

Most melanocytes are found in the basal layer of the epidermis and hair follicles. Their homeostasis is regulated by epidermal keratinocytes. Some melanocytes reside in the dermis and can be found surrounding the sebaceous glands or near the lactiferous ducts of the nipples [7]. Melanocytes function solely to produce melanin. In human skin, the main function of melanin is to protect against the toxic and carcinogenic effects of ultraviolet radiation (UVR) [8]. Consequently, melanocytes may be particularly susceptible to genetic mutations and need to be regularly renewed to prevent the accumulation of DNA damage [8].

In response to environmental triggers and mitogenic stimuli, keratinocytes secrete factors that regulate the survival, differentiation, proliferation, and mobility of melanocytes. Keratinocytes also stimulate melanocytes to produce melanin to protect the skin from the potentially harmful effects of UVR. In addition, depending on their anatomic location, some melanocytes are subject to the effects of steroid hormones due to the expression of nuclear estrogen and androgen receptors [9]. Estrogens may alter cellular function through the classical pathway, which depends on the interaction between estrogen and nuclear receptors. Estrogens can also act through the non-classical pathway, which depends on the capacity of estrogen to interact with estrogen receptors in the cell membrane and with non-steroidal hormone receptors such as the G protein-coupled receptor 30 (GPR30). The non-classical pathway is faster and activates the mitogen-activated protein kinase (MAPK) pathway, thus activating gene transcription [9].

Therefore, adult epidermal melanocytes may proliferate in response to growth factors released by keratinocytes during the process of skin regeneration and by other mitogenic stimuli, such as the interaction of estrogen with other signaling pathways. This proliferation can be lentiginous (solitary melanocytes along the dermal-epidermal interface), occur in clusters or nests (clusters of melanocytes in the dermal-epidermal junction and/or in the dermis), or be pagetoid (solitary melanocytic cells throughout the epidermis) [8].

Damaged melanocytic cells are presumably reabsorbed in the epidermis, although the movement of melanocytes in the epidermis is imperceptible. Melanocytes have been noted in higher layers of the epidermis (pagetoid melanocytes) following acute UV exposure [10]. These cells are relatively large, clear, polydendritic, and deficient in tonofilaments and desmosomes. They can be distinguished by the presence of spherical or ovoid cytoplasmic organelles that contain melanin granules and are bounded by a dense membrane, called the melanosome, which extends into the dendrites [8,11].

The pigment in melanosomes is transported from the dendrites to the keratinocytes through mechanisms that are not yet well defined [11]. The number of skin melanocytes is relatively constant, and thus, variations in the number, size, composition, and distribution of melanosomes in the epidermis determine differences in skin pigmentation [8,11].

Epidermal melanocytes are located among the keratinocytes of the basal layer; like the keratinocytes, they are in contact with the dermis. The dendritic extension of each melanocyte is in close contact with keratinocytes from the basal and adjacent layers. This arrangement serves to maximize the volume of tissue that the melanocyte can supply with melanin and forms an epidermal melanin unit [8,11].

In the epidermis, a unit of pigmentation is formed by epidermal melanocytes interacting with 36 to 40 keratinocytes, whereas melanocytes in the basal layer and follicular melanocytes interact with 4 to 5 keratinocytes. The equilibrium of melanocytes in the epidermal melanin unit is maintained through the regulation of cell division [8,11].

Skin surface expansion occurs from childhood through adulthood, and melanocytes proliferate to maintain the appropriate proportion of melanocytes to keratinocytes in the basal layer [10]. In adult humans, melanocytes enter the G0 phase of the cell cycle and are able to proliferate when necessary, although at a lower rate. Melanocyte proliferation requires decoupling with keratinocytes, loss of the dendrites, cell division, and migration throughout the basal layer, followed by recoupling with keratinocytes to re-form the epidermal melanin unit [11-13]. How melanocytes and keratinocytes are able to maintain this equilibrium, which is only disrupted in certain locations with the development of a nevus or a melanoma, is poorly understood [12].

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Epidermal melanocytes are present in all regions of the body. The density of melanocytes varies regionally from 500 to 2000 cells/$m^2$ of skin surface. Skin from the genital areas and face have the maximum melanocyte density (2000 cells/$m^2$ of skin surface), and skin from the trunk and arms has lower melanocyte densities (800 to 1000 cells/$m^2$ of skin surface) [10]. With increasing age, there is a reduction in the population of melanocytes and a decline in melanin synthesis [6,11-13].

Human skin has a high capacity for regeneration and, on average, is capable of generating new cells every 28 days. These cells undergo re-pigmentation by melanocytes. During this process, signaling pathways and transcription factors regulating adult melanin systems are similar to those in the embryo, and it is through these homeostatic signaling pathways that appropriate numbers of melanocytes are maintained [8,12,14].

Therefore, cell cycle regulation is key for the development of normal melanocytes, both during embryogenesis and cell regeneration. Deregulation of the cell cycle and its components may result in uncontrolled cell proliferation and the formation of melanocytic tumors.

Melanocytes are derived from pluripotent and transient stem cells of the dorsal neural crest. Under normal conditions, these embryonic cells are exposed to appropriate signaling pathways and transcription factors and migrate to specific locations within the embryo, where they complete the process of differentiation to form melanocytes [8,15].

The choice between a ventral or dorsal migration route occurs when undifferentiated cells begin to express specific markers, such as transcription factors, for the activation and/or repression of cell growth [16]. Therefore, neural crest cells of the melanogenic lineage adopt the genetic and morphological expression profiles of melanocytes during the development or specification of melanoblasts [16].

Pluripotent stem cells initially express activator transcription factors, such as SOX10 [SOX gene family/high mobility group (HMG) box], PAX3 (paired box gene family), and the repressor transcription factor FOXD3 (forkhead protein gene family), which plays the central role in determining the fate of melanocytes by suppressing melanogenesis in the pre-migratory cells of the neural crest. These cells divide and give rise to originate bipotent neural/glial and glial/melanogenic precursor cells. Finally, the glial/melanogenic precursor cells divide to generate melanoblasts [12,16].

PAX3 is expressed in the majority of melanocytic nevi and melanomas. Recently, it has also been identified in normal skin melanocytes [16], which suggest that, in addition to regulating the undifferentiated state, PAX3 may act on normal skin melanocytes. This factor appears to be necessary for the cellular response to environmental mitogenic stimuli and may thus play a role in melanocyte proliferation [17].

After emerging from the neural tube, melanogenic precursor cells initiate the expression of the microphthalmia-associated transcription factor (MITF) and other melanoblast markers, such as tyrosinase-related protein 1 (Tyrp-1) and dopachrome tautomerase (Dct or tyrosinase-related protein-2). MITF is essential for the formation of normal melanocytes during embryonic development, in addition to regulating the expression of several essential genes for melanogenesis, such as BCl2 (B-cell lymphoma-2), Tyrp-1, Dct, c-Kit (a proto-oncogene), and melanocortin-1 receptor gene (MCr-1). The melanocyte-stimulating hormone alpha-MSH is the ligand for MCr-1. When activated, MCr-1 stimulates adenylate cyclase conversion from adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). This process, in turn, causes the phosphorylation of CREB (cAMP-responsive element-binding protein) and induces MITF gene transcription. MITF expression causes its own translocation to the cytoplasm, where it is phosphorylated by ERK (extracellular signal-regulated kinase), which is itself activated by activated c-Kit. This phosphorylation causes MITF degradation and the transcription of other effector genes, such as BCl-2, TYR, and p16 [14]. A mutation in any of these genes results in failure of normal melanoblast development; consequently, the risk of melanocytic tumors may increase [12,14,16,17].

When invading the ectoderm, melanoblasts differentiate into melanocytes and express the melanogenic enzyme tyrosinase. Melanocytes can be detected in the epidermis at approximately the 50$^{th}$ day of intrauterine life [16,17]. Therefore, the survival, migration, and correct epidermal inclusion of melanocytes during embryogenesis are dependent on interactions between specific cell surface receptors and their extracellular ligands. However, the location and timing of melanoblast specification remains controversial.

Most neural crest cells are believed to undergo specification before migration, with only a few remaining pluripotent. The cells that remain pluripotent are likely the stem cells that persist into adulthood [18]. The persistence of immature melanocyte precursors has recently been demonstrated in the bulge region of hair follicles in adult skin [19].

Different signaling pathways play important roles in the migration, survival, and epidermal inclusion of melanocytes in both the embryonic and adult melanin systems [8,15]. These pathways can be either dependent on or independent of c-kit and its stem cell factor (SCF) ligand. As previously described, c-kit can directly or indirectly phosphorylate MITF through ERK phosphorylation.

In adult skin melanocytes, the c-kit receptor and its SCF ligand are the primary regulators of homeostasis; epidermal keratinocytes, endothelial cells, and fibroblasts are responsible for the production of SCF [8,12]. Increased or decreased SCF/Kit expression causes the proliferation or loss of melanocytes, respectively [8].

The RAS (proto-oncogene serine/threonine-protein kinase) signaling pathway is also important in determining the survival and proliferation of melanocytes. The activation of this pathway occurs through kinase receptors and their ligands, which are proteins that are released by various types of cells and that act as growth factors. Two other signaling pathways are initiated by RAS pathway activation: MAPK (mitogen-actived protein kinases) and PI3K/AKT (phosphatidylinositol 3-kinase).

Signals occurring in the MAPK pathway are responsible for inducing cell proliferation. RAS activates BRAF (proto-oncogene B-Raf) through phosphorylation, and activated BRAF phosphorylates and activates MEK (MAP kinase activator), which then activates ERK. The translocation of activated ERK to the nucleus leads to cell proliferation through the activation of the cyclin complex D1-CDK4/6. This complex phosphorylates the retinoblastoma (Rb) protein, which is located in the nucleus of the E2F (gene regulator protein)/Rb complex and, consequently, activates E2F. E2F regulates protein transcription, allowing the cell to overcome the G1 restriction point [14].

The role of UVR in the induction of BRAF mutations is controversial because most commonly acquired melanocytic nevi can develop this mutation [19], as can congenital melanocytic nevi [20] and melanocytic nevi located in sites that are not exposed to UVR [21], such as atypical genital melanocytic nevi [20].

Presumably, among the various clonal events, polyclonal BRAF mutations may be the initial event in melanocyte transformation during the development of melanocytic tumors [22], according to the anatomical location.

The expression of protein 53 (p53) is inhibited by binding to MDM2 (mouse double minute-2), which promotes the ubiquitination of p53. DNA damage triggers p53 phosphorylation, inhibiting MDM2 binding and consequently increasing p53 expression. In response to cell damage, p53 activates the cyclin-dependent kinase inhibitor 1a (CDKN1A or p21), blocking the cell cycle by inhibiting the cyclin complex D1-CDK4/6 and transcription of the Rb pathway. Furthermore, p53 promotes cell cycle blocks and increases apoptosis in response to DNA damage by activating the transcription of genes encoding BAX proteins, which promote the release of cytochrome c from the mitochondria. The activation of p53 also inhibits the transcription of genes that encode BCl2 proteins, which block the release of cytochrome c from the mitochondria [14].

Apoptosis can be activated through intracellular (mitochondrial) pathways or extracellular (via cell surface receptor/FAS) death signals. The extracellular pathway leads to intracellular aggregation of molecules and caspase activation [14].

The gene located in the CDKN2A locus of the 9p21 chromosome has an unusual structure because it has two different protein transcripts: p16 (or INK4A) and p14 (or ARF – alternative reading frame), both of which are cell cycle inhibitors. The p16 protein binds to cyclin D1 and inhibits the activation of CDK4/6, promoting the dephosphorylation of Rb. Rb inhibits cell cycle progression by controlling the transcription of the E2F gene. Rb phosphorylation decreases its affinity for E2F, and free E2F promotes its own transcription. The loss of p16 functionality causes a lack of constraint on Rb phosphorylation, the freeing of E2F, and uncontrolled cell growth [14].

The p14 protein prevents p53 degradation by sequestering MDM2 and inhibiting the binding of MDM2 to p53. The high levels of p14 stabilize p53 and allow the induction of p21, a cell cycle inhibitor, by blocking Rb phosphorylation. The lack of p14 causes the ubiquitination and degradation of p53, removing the cell cycle blockage and leading to the hyperphosphorylation of Rb and the progression of the cell cycle [14].

Aberrations in cell cycle control underlie melanocytic tumor formation, and among the most common changes in cell cycle control are those that affect the CDKN2A gene locus, which ultimately controls Rb and p53 [14].

The PI3K/AKT pathway is also important in the regulation of cell proliferation and survival; however, the relationship between this pathway and the survival of skin melanocytes is poorly understood. PI3K (phosphoinositide 3-kinase) is a lipid kinase responsible for the activation of AKT (protein kinase B – PKB). The inhibitory effect of this pathway is exerted by the phosphatase and tensin homolog (PTEN), which dephosphorylates phosphatidylinositol bisphosphate (PIP2) [14]. PDK1 (phosphoinositide-dependent kinase) also phosphorylates AKT [14]. The balance between AKT activation and the regulatory activity of PTEN is a key determinant of cell cycle progression. Several mitogenic processes are activated through the activation of AKT, including the inhibition of apoptosis, cell growth, and proliferation [14].

Activated AKT can inhibit TSC2 (tuberous sclerosis protein 2) and can activate mTORC2 (mammalian target of rapamycin C2), leading to cell proliferation. The TOR pathway is also activated by RAS and Rheb (Ras homolog enriched in brain) signaling; however, the role of RAS in mTORC2 activation remains unclear. Rheb negatively regulates BRAF and is inhibited by TSC [14]. Therefore, activating mutations in the MAPK pathway may lead to melanocytic proliferation and interact with different proteins from other pathways.

The Wnt (Wingless-type mouse mammary tumor virus integration site member)/β-catenin pathway also plays an important role in embryogenesis and tissue homeostasis. This pathway is involved with the migration, development, and differentiation of melanoblasts into melanocytes through the activation of the Frizzled receptor. In the presence of Wnt signals, β-catenin phosphorylation is inhibited by a complex and poorly understood mechanism. A protein called "Dishevelled" is likely to inhibit the activity of glycogen synthase kinase (GSK–3). Due to this inhibition, β-catenin is not targeted for degradation by proteasomes. It accumulates in the cytoplasm and can migrate to the nucleus, interacting with and activating the transcription factors of a group of genes (c-Myc, cyclin D1, and MITF) that are responsible for cell proliferation and differentiation [22,23]. The Wnt/β-catenin pathway seems to be associated with the pathogenesis of melanocytic nevi and melanoma because almost all melanocytic nevi are positive for nuclear β-catenin. In melanoma, loss of the Wnt/β-catenin signaling pathway is associated with increased cell motility and invasive capacity of the tumor [24,25]. However, further studies are needed to elucidate the role that the loss of signaling plays in this complex mechanism.

Not surprisingly, the abnormal growth of melanocytes during the development of melanocytic tumors recapitulates several critical steps in the normal development of melanocytes. However, the degree of melanocyte differentiation and plasticity in melanocytic tumors remains unknown, given that the state of the origin cells in nevi and melanomas has not been demonstrated. Furthermore, there are location-dependent differences in the proliferative potential of melanocytes in response to mitogenic stimuli.

Stem cells are believed to be involved in this process due to the possible clonal origin, multiple genetic factors (such as polymorphism of the MCR-1 gene, melanocyte resistance to apoptosis, and polyclonal mutations of BRAF), and the presence of triggering factors or mitogens, especially UVR. Understanding the molecular changes that involve important cellular processes, such as the cell signaling network, cell cycle regulation, and cell death, is essential to better understand the formation of melanocytic tumors and to understand why some growths remain nevi and others transform into melanomas.

Answering these questions opens possibilities for future research into the development of melanocytic tumors that will be aimed at better understanding the molecular interactions of melanocytes with their niche, the dependence of melanocyte development on anatomical location, and the process of skin regeneration.

## References

1. Yarak S, Ogawa MM, Hirata S, de Almeida FA (2010) Prevalence of acquired melanocytic naevi in Brazilian schoolchildren. Clin Exp Dermatol 35: 581–587.
2. Autier P, Boniol M, Severi G, Giles G, Cattaruzza MS, et al. (2001) The body site distribution of melanocytic naevi in 6–7 year old European children. Melanoma Res 11: 123–131.
3. Harrison SL, Buettner PG, MacLennan R (1999) Body-site distribution of melanocytic nevi in young Australian children. Arch Dermatol 135: 47–52.
4. Green A (1992) A theory of site distribution of melanoma: Queensland, Australia. Cancer Causes Control 3: 513–516.

5. Krengel S (2005) Nevogenesis--new thoughts regarding a classical problem. Am J Dermatopathol 27: 456–465.

6. Ross AL, Sanchez MI, Grichnik JM (2011) Molecular nevogenesis. Dermatol Res Pract, Article ID 463184.

7. Bolognia JL, Orlow SJ (2008) In: Bolognia J, Jorizzo J, Rapini R (eds). Biology of Melanocytes. Dermatology Edition: Text with Continually Updated Online Reference. (2ndedn). Mosby Elsevier, New York, 935–945.

8. Grichnik JM (2008) Melanoma, nevogenesis, and stem cell biology. J Invest Dermatol 128: 2365–2380.

9. Verdier-Sevrain S, Bonté F, Gilchrest B (2006) Biology of estrogens in skin: implications for skin aging. Exp Dermatol 15: 83–94.

10. Petronic-Rosic V, Shea CR, Krausz T (2004) Pagetoid melanocytosis: when is it significant? Pathology 36: 435–444.

11. Herlyn M, Berking C, Li G, Satyamoorthy K (2000) Lessons from melanocyte development for understanding the biological events in naevus and melanoma formation. Melanoma Res 10: 303–312.

12. White MR, Zon LL (2008) Melanocytes in development, regeneration, and cancer. Cell Stem Cell 3: 242–252.

13. Ross AL, Sanchez MI, Grichnik JM (2011) Nevus senescence. ISRN Dermatol Article ID 642157, 8 pages

14. Ibrahim N, Haluska FG (2009) Molecular pathogenesis of cutaneous melanocytic neoplasms. Annu Rev Pathol 4: 551–579.

15. Dupin E, Le Douarin NM (2003) Development of melanocyte precursors from vertebrate neural crest. Oncogenes 22: 3016–3023.

16. Thomas AJ, Erickson CA (2008) The making of a melanocyte: the specification of melanoblasts from the neural crest. Pigment Cell Melanoma Res 21: 598–610.

17. Medic S, Ziman M (2010) PAX3 expression in normal skin melanocytes and melanocytic lesions (naevi and melanomas). PLoS One 5: e9977.

18. Delfino-Machin M, Chipperfield TR, Rodrigues FS, Kelsh RN (2007) The proliferating field of neural crest stem cells. Dev Dyn 236: 3242–3254.

19. Teng L, Labosky PA (2006) Neural crest stem cells. Adv Exp Med Biol 589: 206–212.

20. Papp T, Pemsel H, Zimmermann R, Bastrop R, Weiss DG, et al. (1999) Mutational analysis of the N-ras, p53, p16(INK4a), CDK4, and MC1R genes in human congenital melanocytic naevi. J Med Genet 36: 610–614.

21. Nguyen LP, Emley A, Wajapeyee N, Green MR, Mahalingam M (2010) BRAF V600E mutation and the tumour suppressor IGFBP7 in atypical genital naevi. Br J Dermatol 162: 677–680.

22. Lin J, Goto Y, Murata H, Sakaizawa K, Uchiyama A, et al. (2011) Polyclonality of BRAF mutations in primary melanoma and the selection of mutant alleles during progression. Br J Cancer 104: 464–468.

23. Widlund HR, Horstmann MA, Price ER, Cui J, Lessnick SL, et al. (2002) Beta-catenin-induced melanoma growth requires the downstream target Microphthalmia-associated transcription factor. J Cell Biol 158: 1079–1087.

24. Bachmann IM, Straume O, Puntervoll HE, Kalvenes MB, Akslen LA (2005) Importance of P-cadherin, beta-catenin, and Wnt5a/frizzled for progression of melanocytic tumors and prognosis in cutaneous melanoma. Clin Cancer Res 11: 8606–8614.

25. Weeraratna AT, Jiang Y, Hostetter G, Rosenblatt K, Duray P, et al. (2002) Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. Cancer Cell 1: 279–288.